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14. ABSTRACT

A technique for accurate measurement of the principle refractive indices and birefringence for silklike samples is presented. It is based on rotating the linear polarization of the illuminating light on a silk immersed in reference liquid to achieve index matching at the silk/liquid interface. The technique was used to measure the principal refractive indices of a P. eburnus radial silk at different strains. This in turn allows the calculation of strain-optic coefficients. The first measurement of the strain-optic coefficients of spider silk is presented. The technique is more

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Block 13: Supplementary Note

Hybrid immersion-polarization method for measuring birefringence applied to spider silks

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A technique for accurate measurement of the principle refractive indices and birefringence for silklike samples is presented. It is based on rotating the linear polarization of the illuminating light on a silk immersed in reference liquid to achieve index matching at the silk/liquid interface. The technique was used to measure the principal refractive indices of a *P. eburnus* radial silk at different strains. This in turn allows the calculation of strain-optic coefficients. The first measurement of the strain-optic coefficients of spider silk is presented. The technique is more generally applicable to strain-optic study of birefringent micro-optic samples. © 2011 Optical Society of America

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The remarkable mechanical properties of spider silk have been well documented and the function of these properties with regard to prey capture is well understood [1–3]. There is now a growing interest in the optical properties of spider silk, and the optics of transparent spider webs more generally [4]. This interest is fuelled by biologists who seek to use the optical properties of spider silk to explain aspects of spider behavior and evolution [5], and physicists who seek to engineer materials that emulate aspects of the silks themselves.

Study of the optical properties of spider silk has largely been restricted to measurements of reflectivity [5], surface quality [6,7], and refractive index, mainly due to the practical difficulties of handling the silks. Spider silks are very fine (diameter = 1–10 μm) and must be kept under tension. This prohibits cleaving or breaking the silk fibers and rules out the use of most available methods for measuring refractive index. Inverse-scattering-based techniques have been used; these are generally limited to an accuracy of ± 0.01 [8–10]. The de Sénarmont method has been applied to measuring birefringence of spider silks [11]. But this method does not give the principal refractive indices of the silk.

A recently developed method for measuring the refractive index of spider silks, dubbed the image contrast immersion method [12], involves immersion of the silk in a series of liquids with calibrated refractive indices and quantifying the visibility of the silk. The refractive index is determined as the minimum in visibility of the silk/liquid interface as a function of refractive index for the standard liquids [13]. An accuracy of ± 0.0005 has been demonstrated using a set of liquids with refractive indices separated by 0.002 and a basic interpolation technique [12].

Previously, birefringence measurements on spider silks were performed using this technique to measure the refractive index when the silk was illuminated with light linearly polarized along the axis of the silk and perpendicular to it. This gave measurements of both principal refractive indices and the birefringence is their difference. The technique outlined in this Letter is a further development of the image contrast immersion method. The method involves rotating the orientation of the incident linear polarization on the immersed silk. There is an orientation where index matching between the silk and liquid is obtained. Once this is repeated for a second liquid, the principal refractive indices of the spider silk are calculated using the method outlined herein. The modified procedure is faster and more accurate compared to that in [12], due to the need to use fewer immersion liquids and the relative simplicity of continuously rotating the orientation of the incident polarization. The drawback of this method is that it can only be used on samples where there are two or more available calibrated immersion liquids that lie between the principal refractive indices. Commercially available sets of immersion liquids typically have intervals of 0.002 [13]. Thus the minimum birefringence that could be measured with these sets is of order 0.003.

This hybrid immersion–polarization method uses the same experimental setup as outlined previously [12]. Light from the metal-halide lamp source was passed through a bandpass filter centered at 589 nm and a linear polarizer that was set in a rotation stage fixed to the microscope assembly.

A number of refractive-index oils (Cargille, series A) with refractive indices in between the principal refractive indices of the spider silk were identified through a trial-and-error approach. The silk was then immersed in a suitable refractive-index oil and the orientation of the polarization (as depicted in Fig. 1) that resulted in index matching between the silk/oil interface was recorded. This was repeated for the second oil, giving two different orientations corresponding to two different oils with a known refractive index. The silk was washed with isopropanol between immersions. Isopropanol did not affect the refractive index of the silk; this was verified

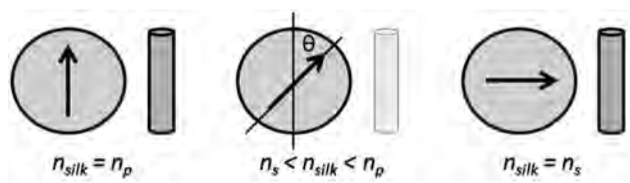

Fig. 1. Polarizer positions for immersed silk illuminated with light polarized parallel (left) and perpendicular (right) to the silk axis. Minimum visibility occurs at some orientation θ (center).

by immersing the silk in the same oil before and after an isopropanol wash and checking that the measured values did not change.

Radial silks can be approximated as uniaxial crystals with the birefringent axis parallel to the axis of the silk. For a given orientation of the incident polarization θ (measured with respect to the silk axis), the refractive index of the silk is given by

$$n_{\mathrm{silk}}(\theta) = \left(\frac{\cos^2\theta}{n_p^2} + \frac{\sin^2\theta}{n_s^2}\right)^{-1/2}, \tag{1}$$

where n_p and n_s are the principal refractive indices of the spider silk for the polarizations oriented parallel and perpendicular to the silk length, respectively. Equation (1) was rearranged to give

$$n_s = \left(\frac{n_p^2 n_{\mathrm{silk}}^2 \sin^2\theta}{n_p^2 - n_{\mathrm{silk}}^2\cos^2\theta}\right)^{1/2}. \tag{2}$$

Refractive-index matching occurred between the silk and oil (where $n_{\mathrm{silk}} = n_{\mathrm{oil}}$) at measured orientations θ_1 and θ_2 for oils with refractive indices n_{oil1} and n_{oil2}, thus,

$$n_s = \left(\frac{n_p^2 n_{\mathrm{oil1}}^2 \sin^2\theta_1}{n_p^2 - n_{\mathrm{oil1}}^2\cos^2\theta_1}\right)^{1/2}, \tag{3}$$

$$n_s = \left(\frac{n_p^2 n_{\mathrm{oil2}}^2 \sin^2\theta_2}{n_p^2 - n_{\mathrm{oil2}}^2\cos^2\theta_2}\right)^{1/2}. \tag{4}$$

To obtain the principal refractive indices, Eq. (3) and (4) were solved simultaneously for n_p:

$$n_p = n_{\mathrm{oil1}} n_{\mathrm{oil2}} \left(\frac{\sin^2\theta_2 - \sin^2\theta_1}{n_{\mathrm{oil2}}^2\sin^2\theta_2 - n_{\mathrm{oil1}}^2\sin^2\theta_1}\right)^{-1/2}. \tag{5}$$

Once n_p was evaluated, n_s was calculated by substitution.

A typical n measurement is shown in Fig. 1. The uncertainty of an n measurement consisted of two contributions. The first contribution was due to the uncertainty in θ. For the silk samples studied, θ could be determined to within an accuracy of $\pm 1°$ by monitoring the intensity profile across the silk image as the polarization was rotated. The second contribution was due to the inhomogeneity of the silk sample and manifested as imperfections in the theoretical fit to experimental data (such as the fit shown in Fig. 2.). At least three oils were needed to assess the uncertainty due to sample inhomogeneity. The more oils that were used, the more accurately the uncertainty could be determined, with up to six used for each n measurement. As the uncertainty due to sample inhomogeneity was larger than the uncertainty due to θ in the specific case of spider silks, the overall uncertainty in the n measurement was determined by applying Eq. (5) to different pairs of oils and calculating the spread of n_p and n_s values that were obtained as a result. Only pairs of oils where the uncertainty due to θ was small ($|\theta_1 – \theta_2| > 10°$) were included in this calculation. In general, the procedure for calculating the uncertainty depends on how the contribution due to θ and sample inhomogeneity compare.

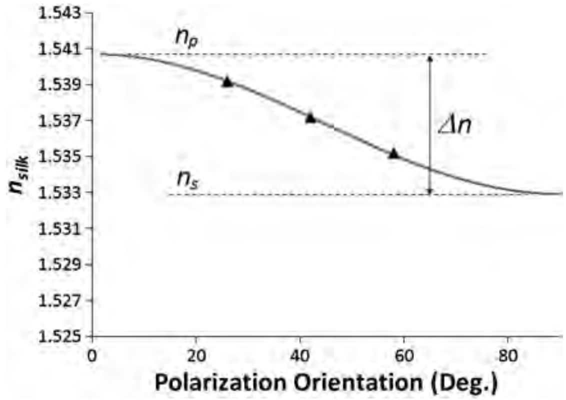

Fig. 2. Typical set of measurements for measuring the principal refractive indices of a radial silk sample. Three oils were used; data points are shown as black triangles, while the gray curve is Eq. (1) with $n_p = 1.5407$ and $n_s = 1.5329$. Uncertainty bars along both axes are smaller than the data points themselves.

The birefringence of a *P. eburnus* radial silk was measured as a function of strain by mounting the silk on a Vernier caliper and repeating the procedure outlined above for different strains, applied by adjusting the distance between the teeth of the caliper. The initial length of the radial silk was 15.00 mm and the silks were initially mounted such that the native tension of the web was preserved. The variability of strain within a single web is probably less than 10% [14]. The silk of *P. eburnus* is not very extensible, though it does contract, keeping the silk fiber taut as the strain is reduced. This is in contrast to most dragline/radial spider silks, which are highly elastic when stretched. The strain was reduced by adjusting the silk to lengths of 14.00, 13.00, and 12.00 mm.

The measured principal refractive indices as a function of strain are shown in Fig. 3. Changes in refractive index were due to the strain-optic effect and changes to the silk structure that occur in response to the applied strain, including density changes and reordering of the nanocrystallite domains [15].

The strain-optic tensor elements are defined as [16]

$$\Delta(1/\kappa_{ij}) = p_{ijkl}\varepsilon_{kl}, \tag{6}$$

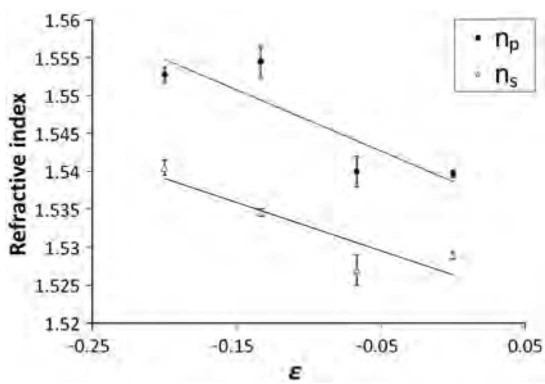

Fig. 3. Measured principal refractive indices of *P. eburnus* radial silk as a function of strain. Straight lines indicate linear fits to the data.

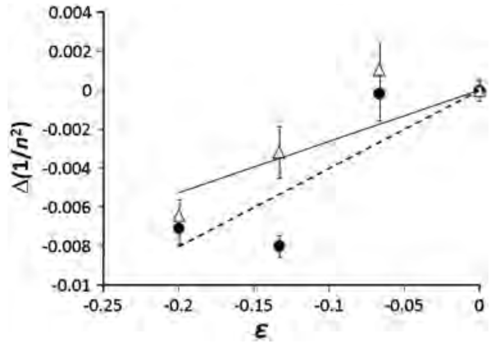

Fig. 4. Measured values for $\Delta(1/n^2)$ as a function of strain for a *P. eburnus* radial silk. Dotted and bold lines indicate linear fits for n_p (black points) and n_s (white points), respectively.

where κ_{ij} is the optical dielectric tensor, p_{ijkl} is the strain-optic tensor, and ε_{kl} is the strain, calculated as $L/L_0 - 1$, where L is the silk length and L_0 is the initial silk length. For a uniaxial strain applied along the axis of a silk thread,

$$\Delta(1/n_p^2) = p_{1111}\varepsilon, \tag{7}$$

$$\Delta(1/n_s^2) = p_{2211}\varepsilon. \tag{8}$$

The measured values for $\Delta(1/n^2)$ as a function of strain are shown in Fig. 4. If we assume that 100% of the refractive-index change is due to the strain-optic effect, a linear fit yields effective strain-optic coefficients of 0.040 ∓ 0.009 for p_{1111} and 0.026 ∓ 0.008 for p_{2211}. A comparison to known values for other optical materials is shown in Table 1. The low values exhibited by the silk possibly reflect the webs' need to maintain their appearance over a wide range of environmental conditions. The real strain-optic coefficients will depend on the contribution to the observed refractive-index change from structural changes within the silk. Also, due to the large applied strain, there are possibly nonlinear effects that have not been accounted for here. Kiesel *et al.* developed a method for characterizing the nonlinear strain-optic response [17]. Applying this to highly extensible spider silks is a subject of future work. An intriguing possibility is spider silk that exhibits very small refractive-index changes over large strains.

In conclusion, we have enhanced a recently developed immersion method for measuring the refractive index of spider silks to allow quick, accurate measurements of the birefringence of *P. eburnus* radial silks at strains between 0 and −0.2. The effective strain-optic coefficients were found to be 0.040 ∓ 0.009 for p_{1111} and 0.026 ∓ 0.008 for p_{2211}. The new technique will be applicable to micrometer diameter optical fibers, like transparent spider silks, and to other micro-optical elements and samples showing birefringence. It represents a new tool for studying strain-optic effects in such birefringent micro-optics.

Table 1. Measured p_{1111} and p_{2211} Values for Different Optical Materials

Material	p_{1111}	p_{2211}
Silk *P. eburnus*	0.040	0.026
Fused silica [18]	0.126	0.260
As_2S_3 [18]	0.24	0.22
Polymethyl methacrylate [19]	0.300	0.297

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